JOURNAL OF AGRICULTURAL RESEARCH

VOL. 66 WASHINGTON, D. C., MARCH 15, 1943 NO. 6

# AN APPARATUS FOR STUDYING RESPIRATION OF AZOTOBACTER IN RELATION TO THE ENERGY INVOLVED IN NITROGEN FIXATION AND ASSIMILATION [1]

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## INTRODUCTION

The discovery of *Azotobacter* by Beijerinck (*1*) [3] in 1901, encouraged a vast amount of work on the physiology on this group of nitrogen-fixing bacteria. Since the free-energy data published by Lewis and Randall (*10*) appeared, the interest of other investigators (*5, 6, 7, 12, 13*) in these organisms has centered on the energetics and efficiency of the nitrogen-fixation process, the efficiency of growth, and also on the efficiency of oxygen utilization.

Studies on the rate of respiration of *Azotobacter*, while being subjected to a wide range of carefully controlled environmental conditions, were started in August 1929, at Berkeley, Calif., and continued during the following 2 years. In this paper the apparatus and methods used are described and certain of the results of the investigations are presented.

The chemical reactions catalyzed by *Azotobacter* to obtain the necessary energy for its metabolic processes vary greatly. Under aerobic conditions the oxidation of glucose to carbon dioxide and water is a typical reaction which is catalyzed by these organisms. It may be written, using the terminology of Lewis and Randall (*10*),

$$C_6H_{12}O_6+6O_2=6CO_2+6H_2O;\ \Delta H=-674{,}000 \text{ cal.}$$

The rate at which this reaction is carried out may be considered a direct measure of the metabolic activity of the culture. Consequently the rate of respiration of a culture of *Azotobacter* under different environmental conditions can be determined by measuring the rate at which this reaction proceeds. This rate may be measured in either of two ways: (1) by measuring the rate of disappearance of the reacting substances, such as the rate of absorption of oxygen; (2) by measuring the rate of formation of the end product, carbon dioxide, or the heat of the reaction.

As long as the organisms are utilizing free oxygen, as opposed to combined oxygen, in the reactions catalyzed for a source of energy, a measure of either the rate of oxygen consumption or the rate of carbon dioxide production or the rate at which heat is evolved will give an accurate index to its metabolic activity.

[1] Received for publication January 12, 1942. This investigation was made possible by the National Research Council.

[2] The author is indebted to G. N. Lewis, Merle Randall, D. R. Hoagland, A. R. Davis, and C. B. Lipman, of the university staff, and to F. C. Steward, of the Department of Botany, Birkbeck College, University of London, for interest shown in the work and for valuable suggestions and criticisms.

[3] Italic numbers in parentheses refer to Literature Cited, p. 248.

However, there is evidence (*2*, *9*) that *Azotobacter* catalyzes energy-liberating reactions not involving free oxygen under anaerobic or partly anaerobic conditions. This has been confirmed by the author. Under such conditions, the rate at which the reaction proceeds does not give a complete measure of the metabolic activity of the culture or an indication of the total energy liberated. Consequently, experiments at low oxygen tensions in which only the rate of free oxygen utilization or the rate of carbon dioxide production is measured would be of little value in determining the efficiency of growth or of oxygen utilization per cell or the efficiency of the nitrogen-fixation process.

## CRITIQUE OF METHODS

Most of the earlier work on the physiology of *Azotobacter* with respect to oxygen utilization and carbon dioxide production was done with a qualitative outlook and was carried out with few if any precautions taken to keep the partial pressure of oxygen and carbon dioxide in the culture constant. Nor was this work done in such a way that the reactions catalyzed by the organisms would proceed at a maximum rate under the conditions imposed. The methods in these respiration studies were in general inadequate to give an accurate picture of the metabolic activity of *Azotobacter* over relatively short periods under carefully controlled conditions. This critique of the methods may be said to be especially valid where respiration studies were conducted with partial pressures of oxygen which deviated considerably from that in air.

It will be desirable at this point to discuss certain methods used by later investigators. A consideration of their results will be postponed until after the discussion of the experimental results presented in this paper.

Bonazzi (*2*) made a study of the oxygen consumption of *Azotobacter*. In one experiment 2,566 cc. of air was confined over 100 cc. of an inoculated culture medium and incubated for 52 days. From table 4, page 339, of Bonazzi's paper, it is evident that the partial pressure of the gases in the system varied greatly. In this experiment, the concentration of $CO_2$ in the air above the culture increased 160-fold during the experiment. At the same time the partial pressure of oxygen decreased to one-twentieth. The composition of the medium with respect to certain ions was similarly changed. There were 44.36 mg. of $CO_2$ dissolved in the 100 cc. of medium at the end of the experiment, making the medium 0.01 molal with respect to carbonic acid. As there were only 0.02 gm. of $K_2HPO_4$ (0.001 molal) to serve as a buffer, this concentration of carbonic acid, which would not be affected much by the other salts present, would bring the pH of the medium to about 5.5. There would result approximately a 300-fold change in the hydrogen-ion concentration.

It is evident that the question of equilibrium was not taken into consideration in these experiments. During the first few hours in such experiments the available oxygen and carbon dioxide concentration might have permitted a rapid aerobic respiration, but the conditions would soon have become more favorable for anaerobic respiration than for aerobic respiration.

Meyerhof and Burk (*13*) and Lineweaver, Burk, and Horner (*11*) have made an extensive study of the physiology of *Azotobacter* by the use of the Warburg technique (*15*).

The total volume of the apparatus used (*4*, *6*) was about 12 to 16 cc. For the removal of carbon dioxide, these investigators relied entirely upon the normal rate of diffusion of the gas from an alkaline medium (buffered usually at pH 7.3) into the confined atmosphere above, aided only by a gentle rocking of the entire apparatus.

The total gas-liquid interface from which the gas exchange must take place was approximately 6 cm.$^2$, or 3 cm.$^2$ of gas-liquid interface per cubic centimeter of medium (*4*). It was then necessary for the carbon dioxide that did reach the gas phase to diffuse down into a small chamber (approximately 0.8 cm. in diameter) containing 0.2 cc. of potassium hydroxide. The area of the interface between the air and potassium hydroxide was extremely small, being approximately 0.5 cm.$^2$ The gas-liquid interface was increased, however, by shaking the manometers at the rate of 120 cycles per minute with an amplitude of 3 cm.

The environment to which Meyerhof and Burk (*13*) and Lineweaver and associates (*11*) subjected the cultures of *Azotobacter* in the Warburg apparatus was carefully controlled and reproducible. It does not follow, however, that the environment with respect to gaseous exchange was such as to allow respiration to proceed at a maximum rate, especially at the higher oxygen pressures.

## EXPERIMENTAL METHODS

### THE CALORIMETER

The apparatus designed and built for these studies was such that the metabolic activity of a culture could be determined accurately by measuring simultaneously the rate of heat and carbon dioxide production of *Azotobacter* over the entire range of oxygen pressures during nitrogen fixation and nitrogen assimilation.

The differential calorimeter built by Randall and Rossini (*14*) formed the nucleus of the apparatus used in these studies.

The calorimeter consisted of three identical units, as shown in figure 1. Each unit was mounted separately on the under side of a steel plate (*A*). A unit consisted of an electric heating coil (*B*), a cooling coil (*C*), a stirrer (*D*), and one leg of a thermal (*F*), all of which fitted down into a 1,500-cc. vacuum flask (*E*). The vacuum flasks for the three units were held in position by placing them in separate heavy copper cans (*G*), which were then bolted to the steel plate (*A*).

Balsa wood blocks (*H*), and cork supports (*I*) were used to insulate the vacuum flasks from the copper cans and the steel plate.

The entire calorimeter was submerged in an oil bath.

In order for *Azotobacter* to grow normally in the vacuum flask of unit 2 of the calorimeter, it was necessary to build the heating and cooling coils of glass. For the heating coil, a length of manganin wire having a resistance of 9 ohms was coiled and drawn through a thin-walled pyrex U tube. The tube was then filled with oil and sealed, care being taken to leave a small air space to allow for expansion. The cooling coil consisted of several loops of thin-walled pyrex tubing.

A metal bearing was designed for the stirrer so that it would turn freely and yet not leak under pressure. Although the bearing was built to withstand a pressure of 30 mm. of mercury without leaking,

the pressure in the calorimeter never varied more than 4 mm. from atmospheric pressure during an experiment. The mouth of the vacuum flask of unit 2 was sealed with pure gum rubber (*L*) to prevent air from passing from the vacuum flask to the copper can. The three-vaned stirrer (*D*), made of pyrex, was electrically driven at a constant rate of 130 r.p.m.

In order to obtain a direct measure of the rise in temperature produced by the bacteria, it was necessary to hold unit 1, which contained one leg of a multiple-junction thermal ($F_{50}$–$F_{20}$)[4], at a constant temperature. A search of the literature was made to find some salt with a transition temperature near 25° C. which would serve to hold unit 1 at a constant temperature. It was found that the transition temperature of $Cu(NO_3)_2 \cdot 6H_2O$ to $Cu(NO_3)_2 \cdot 3H_2O$ is 25.28° C.

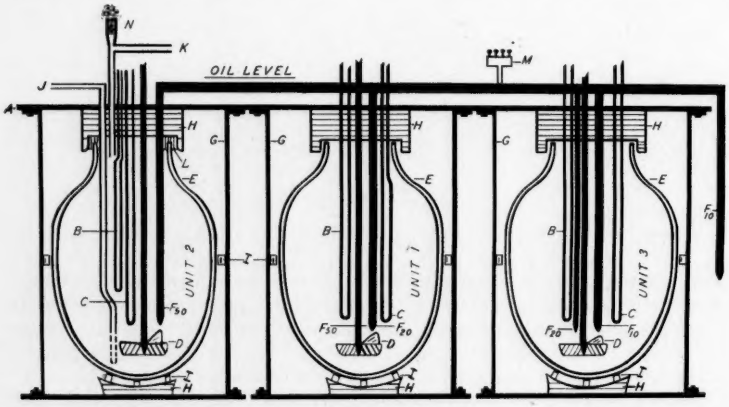


FIGURE 1.—Cross section of the reconstructed calorimeter: *A*, Steel plate; *B*, electric heating coil; *C*, cooling coil; *D*, stirrer; *E*, vacuum flask; *F*, thermals; *G*, heavy copper cans; *H*, balsa wood blocks; *I*, cork supports; *J*, pyrex glass air inlet tube; *K*, exit tube; *L*, gum rubber seal; *M*, terminals; *N*, culture medium inlet.

Other properties of the salt made it ideal for this purpose. Consequently, to the vacuum flask of unit 1, containing 1,000 cc. of a saturated solution of copper nitrate at 25.28° C., was added 800 gm. of an equal mixture of $Cu(NO_3)_2 \cdot 6H_2O$ and $Cu(NO_3)_2 \cdot 3H_2O$. The stirrer of unit 1 was permanently disconnected to prevent the addition of heat to the unit by this means. The oil bath in which the whole assembly was submerged was maintained at 25.28° C. Consequently, no temperature gradient existed between unit 1 and the bath.

Unit 3 of the calorimeter served indirectly to determine the temperature difference between unit 1 and the bath. Consequently, a slight drift in temperature of this unit was not objectionable. Unit 3 was filled with 1,200 cc. of water and at the beginning of each experiment was brought to the temperature of the bath and the stirrer was disconnected. Observations revealed that unit 3 would not vary as much as 0.005° C. for a period of a week. The slight temperature

[4] When the potential difference between units 1 and 2 was measured, the thermal *F* in unit 1 served as one leg of a 50-junction thermal. However, when the potential difference between units 1 and 3 was measured, it served as one leg of a 20-junction thermal.

variations of the oil bath were practically eliminated before they could affect the temperature of the contents of the vacuum flasks of the three units. Unit 3 and the oil bath each contained one leg of a 10-junction thermal ($F_{10}$). By connecting the potentiometer with the proper terminals (*M*), the temperature difference between units 1 and 2, 1 and 3, or 3 and the oil bath, could be accurately determined at any time. Frequent readings of the 20- and 10-junction thermals showed that the temperature drift of units 1 and 3 was insignificant. The drift in temperature of unit 1 was also determined with the aid of a platinum resistance thermometer which was inserted directly into the unit. The maximum drift, as shown by these two separate methods, was never as much as 0.1 percent of the change produced by the culture over a 5-hour period.

### AERATION (GAS-LIQUID INTERFACE)

For *Azotobacter* to carry on its metabolic activities at the maximum rate under the conditions imposed upon it, special precautions were

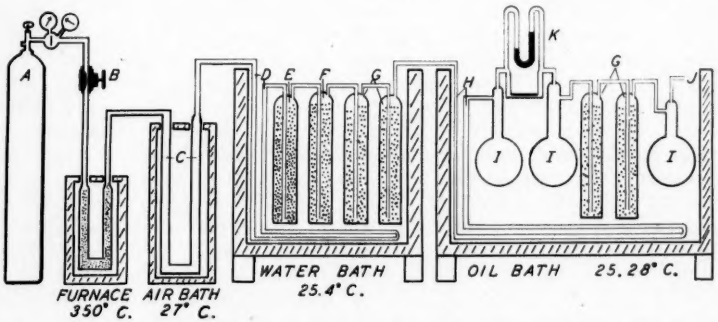


FIGURE 2.—The apparatus used to condition the gas before it entered the calorimeter: *A*, Compressed air tank; *B*, needle valve; *C*, flattened copper pipe; *D*, 15-foot copper tube; *E*, acid tower; *F*, alkali tower; *G*, towers containing distilled water; *H*, copper tube in oil bath; *I*, flasks serving as air cushions; *J*, pyrex tube leading to air inlet *J* of figure 1; *K*, calibrated flow meter.

necessary in order to be certain that the system was in equilibrium with respect to oxygen and that the $CO_2$ was removed as fast as it was formed. Vigorous aeration of the mechanically stirred culture with small bubbles of the gas is not only effective in sweeping out $CO_2$ but also furnishes an extremely large gas-liquid interface from which the gas exchange may take place.

Since heat measurements were made on the culture while it was being vigorously aerated, extreme care had to be taken to condition the air or gas properly before it entered the calorimeter in order that the heat change in the calorimeter due to this variable would be small and constant. The apparatus used to properly condition the air is shown in figure 2.

Compressed air tanks (*A*) were pumped to 1,800 pounds pressure with carbon dioxide-free air. The air from these tanks was forced at a constant rate through a 4-foot pyrex tube with the aid of a reducing valve and a specially designed needle valve (*B*). This tube, 45 mm.

in diameter and containing copper oxide, was heated in a furnace to 350° C. to insure the removal of all traces of combustible gases.

The air was further conditioned by passing it in succession through three adjoining constant temperature baths; the first, an air bath, the second a water bath, and finally the oil bath in which the calorimeter (fig. 1) was also submerged. The air, on leaving the furnace, first passed through a flattened copper pipe (fig. 2, *C*), the temperature of which was held at approximately 27° C. by means of the air bath. After this, it passed successively through a 15-foot copper tube (*D*), and acid tower (*E*), and alkali tower (*F*), and two towers (*G*) which contained distilled water. These were all submerged in the water bath at 25.4° C. The air, on leaving the saturating towers in the water bath, was conducted into the oil bath where it took on the final temperature by passing through 31 feet of copper tubing (*H*) immersed in the oil bath. This bath was kept at 25.28° C. and seldom varied 0.01°.

Since the beads in the saturating towers (*G*) tended to cause an irregular flow of air through the flow meter (*K*) [5] and the culture, large flasks (*I*) which served as air cushions were inserted on both sides of the saturating towers (*G*) in the oil bath. The air, on leaving the copper coil (*H*), passed successively into a 4-liter air cushion (*I*), through a calibrated flow meter (*K*), into a 3-liter air cushion (*I*), and finally into the two large bead towers (*G*) which contained distilled water. The air, after having been brought to the proper temperature, took on the last traces of moisture before passing into a 2-liter air cushion (*I*) and then into the vacuum flask (*E* of unit 2, fig. 1) containing the growing culture. The air was conducted to the bottom of the vacuum culture flask by means of the pyrex tube (*J* of unit 2, fig. 1) and forced its way into the medium directly opposite a three-vaned stirrer, through 20 holes in the side of the tube. The holes were spaced evenly over the lower 4 cm. of the tube. The upper holes were approximately 0.4 mm. in diameter, while the center and lower holes were approximately 0.5 mm. and 0.6 mm. in diameter, respectively.

The stirrer was so effective that thermal equilibrium was maintained constantly. The diameter of the circle described by the stirrer was one-third the maximum diameter of the flask. The stirrer moved 8 cc. of liquid per revolution per vane, or approximately 3,000 cc. per minute. The stirrer passed within 3 mm. of the tube delivering the gas. With more than two revolutions per second, six vanes would sweep by the small openings delivering the gas each second. This was very effective not only in breaking up the bubbles but also in distributing them uniformly throughout the medium. It was estimated from observation that between 50 and 75 percent of the bubbles were broken by the cutting action of the stirrer and the vigorous agitation of the medium.

The surface area of the gas supplied by the different rates of aeration was determined by experiment. Air was forced at a constant rate through a 0.5-mm. opening in the side of a glass tube into a vessel containing 1,200 cc. of water. The number of bubbles was counted for a

[5] The flow meter was calibrated by connecting a gas meter to the end of the assembled line. Five determinations of 1 hour each were made covering the range from 9 to 24 liters per hour. These values were plotted and a straight line obtained.

definite period and the total volume noted. The average volume of the bubbles was determined for eight different rates of aeration between 10 and 24 liters per hour. The volume of the bubbles for the different rates of aeration varied from 0.108 cc. per bubble for a rate of 10.8 liters per hour to 0.095 cc. per bubble for 24 litters per hour, with an average of 0.1019 cc. per bubble. The total surface area of gas available to the liquid for gaseous exchange was calculated for the different rates of aeration, using 0.1019 cc. for the average volume of the bubble and the conservative estimate that 50 percent of the bubbles were broken by the cutting action of the stirrer and the vigorous agitation of the liquid. The gas surfaces are not only extremely large but also present a very efficient surface for gas exchange to take place.

The surface area of liquid coming in direct contact with the gas is still of a higher order of magnitude because of the fact that each bubble of gas having an average surface area of 1.055 cm.$^2$ rising a distance of 15 cm. (25 diameters) through the liquid would come into

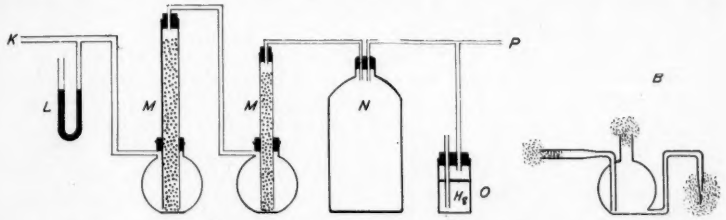


FIGURE 3.—At left, the $CO_2$ absorbing towers and automatic pressure-regulating apparatus: *K*, inlet tube for air from the growing culture in the calorimeter; *L*, water manometer; *M*, $CO_2$ absorbing towers; *N*, carboy serving as air cushion; *O*, mercury pressure-regulating device; *P*, end of air line. At right (*B*), flask used to aerate culture with a side arm for aseptic transfer of the culture to the calorimeter.

direct contact with approximately 25 cm.$^2$ of liquid surface. Consequently, the surface area of liquid exposed to the gas surface varied from $29 \times 10^5$ cm.$^2$ for an aeration rate of 10 liters per hour to $48 \times 10^5$ cm.$^2$ of liquid surface for 24 liters per hour.

The surface of the liquid, being changed continuously by the rapid stirring and vigorous aeration, formed an additional effective gas-liquid interface. Calculations show that the agitation of the liquid by the stirrer alone would move the liquid at a rate sufficient to change the entire surface of the liquid to a depth of 5 mm. 75 times per minute. The air above the culture was completely replaced once every minute with an aeration rate of 18 liters per hour.

### CARBON DIOXIDE ANALYSIS

The air on leaving the growing culture in the calorimeter was drawn by suction through two $CO_2$ absorbing towers which are shown in figure 3, *M*.

Atmospheric pressure, as measured by a water manometer (*L*), was maintained inside the vacuum culture flask despite the number of

towers and flasks in the system. This was accomplished by connecting the end of the air line (*P*) to a constant source of vacuum in series with a 40-liter carboy (*N*) which served as an air cushion. The mercury pressure-regulating device (*O*) automatically maintained the proper negative pressure at all times. The water manometer (*L*) was inserted in the line (fig. 1, *K*) on leaving the culture flask. With a constant pressure from the compressed air tanks forcing the air to the culture flask, and with a constant negative pressure drawing the air from it through the $CO_2$ towers, it was an easy matter to maintain atmospheric pressure in the culture flask well within 4 mm. of mercury at all times. However, when heat measurements were made, even greater care was taken and the pressure was controlled to within 1mm. of pressure. This was found to be necessary inasmuch as the heat correction for the air varied slightly with the pressure.

The $CO_2$ produced by the culture was absorbed in the first tower. To make certain that this absorption tower completely removed all $CO_2$, the air was drawn through a second smaller glass bead tower. The first tower was found to be very efficient in removing all traces of $CO_2$ from the air since the second tower was used continuously for 200 hours without becoming cloudy.

A weighed amount of barium hydroxide solution was added to the assembled $CO_2$-free tower and enough $CO_2$-free water was added so that absorption took place the entire length of the tower. In analyzing for the $CO_2$ present, the tower was disconnected from the line, the beads and alkali in the tower were washed down into the extraction flask, and the tower was thoroughly rinsed with $CO_2$-free water. The excess barium hydroxide was then titrated against 0.08 normal hydrochloric acid, with phenolphthalein as an indicator.

To prepare the tower for absorption, the beads were transferred to a porcelain sieve. The beads, tower, and flask were washed with hydrochloric acid, thoroughly rinsed with tap water, with distilled water, and finally with $CO_2$-free water. The tower was then assembled, the beads added, and immediately sealed. When the barium hydroxide was added, it remained clear, showing that the tower was free from $CO_2$.

### PREPARATION OF CULTURES

Pure cultures of *Azotobacter chroococcum* and *A. vinelandii* were obtained from the American Type Culture Collection.[6] Although the latter was used in most of the work, experiments showed that both gave the same results. In order to be certain that the culture remained pure and did not acquire any unusual characteristics, a second culture of *A. vinelandii* was obtained from the same source 1 year later and carried through the same experiments.

The stock cultures were preserved on dextrose agar slants kept in the ice box. One hundred cubic centimeters of liquid medium in 250-cc. Erlenmeyer flasks was inoculated from the slants. These liquid cultures, incubated at 28° C. and transferred every 2 weeks, were used to inoculate the medium used in the experiments. Every 3 months new liquid cultures were started from the stock cultures kept on ice.

[6] American Type Culture Collection, Chicago, Ill.

The medium used had the following basic composition:

| | |
|---|---|
| $K_2HPO_4$ | 1.0 gm. |
| $MgSO_4.7H_2O$ | 0.2 gm. |
| NaCl | 0.2 gm. |
| $CaCO_3$ | 0.005 gm. |
| $FeCl_3$ | Trace |
| $MnCl_2$ | Trace |
| Glucose | 10 gm. |
| Distilled water | 1,200 cc. |

(The pH was adjusted to 7.1 to 7.2)

The inorganic constituents were added to the distilled water and sterilized in the autoclave at 20 pounds pressure for 30 minutes. Ten grams of glucose were then added and the total weight of medium was made up to 1,195 gm. The medium was then transferred to a sterile flask (fig. 3, *B*) in which the cultures could be aerated with sterile air. This sterile flask, after receiving the adjusted and partly sterilized medium, was autoclaved for 1 hour at 5 pounds pressure.

After the last sterilization, the medium was inoculated with 5 cc. from the liquid cultures, making a total of 1,200 gm. of culture medium. Depending upon the experiment, the content of the flask was either transferred to the sterile vacuum flask (fig. 1, unit 2) immediately, or else inoculated and aerated with sterile air for 12 to 24 hours at the rate of 20 liters per hour before being transferred to the vacuum flask of the calorimeter.

The aseptic transfer of the medium was accomplished with the aid of the side arm (fig. 3, *B*). The tip of the side arm was placed directly over the entrance tube (fig. 1, *N*). The cotton plug and the cotton enveloping of the side arm were flamed and the flask was lowered until the plug and covering of the side arm came in contact before the flame was extinguished. The flask was then lowered still further, allowing the sharp tip to pierce the cotton covering and also the plug and enter the tube (fig. 1, *N*) of the calorimeter. Sterile air forced the medium into the side arm, through the opening in the side of the tube, figure 3, *B*, and into the sterile calorimeter.

The sterilization of unit 2 (fig. 1) of the assembled calorimeter was effectively accomplished by completely filling it and the entrance and exit tubes with a suspension of calcium hypochlorite. This was stirred from 12 to 24 hours and then siphoned out. The remaining hypochlorite was effectively removed by aseptically filling and draining the calorimeter, together with the entrance and exit tubes, four successive times with sterile distilled water, flasks like that in figure 3, *B*, being used.

After this method of sterilizing unit 2 of the calorimeter was adopted, no contamination was found. All the experiments reported were carried to completion with no evidence of contamination.

To be certain that no contaminating organism was present, cultures were taken from the vacuum culture flask at the end of each experiment, transferred to a sterile medium, and also examined microscopically. Subcultures were also made and examined.

## CALIBRATION RESULTS

### HEAT CAPACITY

After the entire apparatus was reconstructed and assembled, calibration runs were made to determine the heat capacity of unit 2 which was to contain the growing culture.

It was found that with the changes in unit 2 (fig. 1) its sensitivity was not affected. Duplicate determinations showed a heat capacity for unit 2 of 64.8 and 64.9 calories per degree. The heat capacity of unit 2 includes that of the vacuum flask, the stirrer, the thermal ($F$), entrance and exit tubes ($J$ and $K$), and the cooling and heating units within the flask.

Calibration runs were made on the sterile medium (1,200 gm.) to determine its heat capacity. Two measurements on the same medium

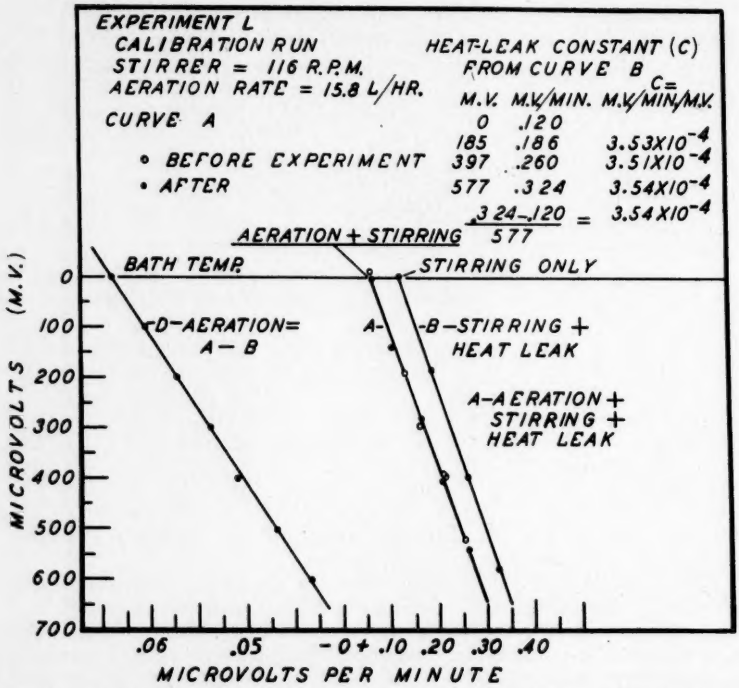


FIGURE 4.—A typical calibration run made to determine the heat-leak constant, stirring correction, and the aeration correction.

gave 1,246.4 and 1,246.5 calories per degree, respectively. The heat capacity of each medium used was determined.

### THE HEAT-LEAK CONSTANT

The heat-leak constant $C$ equals the calories of heat taken up by the calorimeter from the bath per minute per degree that the calorimeter is below the temperature of the bath.

The heat-leak constant of unit 2 was found to have a slightly lower value than that found by Randall and Rossini (*14*). It was reduced by substituting glass for the copper heating and cooling units, by

eliminating evaporation, and by reducing the heat leak around the stirrer by the installation of the airtight bearing.

The heat-leak constant in all experiments was extremely low, ranging from $2.5\times10^{-4}$ to $4.0\times10^{-4}$ microvolts[7] per minute per microvolt below the bath temperature. When converted, a typical heat-leak constant was 0.0486 calorie per minute per unit of 0.10° C. below the bath temperature. Figure 4 shows a typical calibration run and the calculations.

### HEAT CORRECTIONS

The amount of heat added to or removed from the system by the stirring and aeration was determined by special experiments. The constant amount of heat added to the system by stirring was approximately 0.078 calorie per minute for a stirring rate of 130 r. p. m.

The slight cooling caused by the air flow was constant for a given temperature inside the calorimeter and increased very slightly as the temperature of the culture increased. For example, in one experiment the correction of the stirring energy was +2.13 calories, the air correction −2.59 calories, and the heat-leak correction +0.81 calorie. This is a net correction of only +0.35 calorie for a period of one-half hour in a total of 57.50 calories. In this case, the net correction is but 0.61 percent of the total change. However, in some runs where the aeration rate was greater, the net correction was slightly higher. Inasmuch as all heat corrections were determined by special experiments which were accurate to within 10 percent, little error was introduced in the final results.

## OPERATION OF CALORIMETER

To measure the heat produced, the culture was cooled from 600 to 900 microvolts (0.4 to 0.6 of a degree) below the bath temperature by circulating cold air through the glass cooling coil (fig. 1, *C*). Liquid air was used to precool the air passing through the cooling coil; consequently the cooling of the culture required not more than 15 minutes. Microvolt readings of the 50-, 20-, and 10-junction thermals were taken every 15 to 30 minutes until the culture reached the temperature of the bath, which required from 2 to 6 hours, depending on the rate of heat production and the degree of cooling. This constituted one run. The microvolt readings were plotted against time. The heat corrections were made and the calories per hour calculated.

## CALCULATION OF DATA

Figure 5 shows in detail the data and calculations of one run which is typical of approximately 400 which were made. It represents the measurements made and the procedure followed to obtain each point (calories per hour) on the curves of the experiments which will be presented. The heat measurements were made continuously during each experiment except for the short period of 15 minutes between each run. The length of runs varied generally from 2 to 6 hours, depending upon the rate of respiration and the degree to which the culture was cooled.

[7] One microvolt is equal to $46.185\times10^{-4}$ degrees difference in temperature.

## SENSITIVITY OF APPARATUS

Measurements presented in figure 6 show that the apparatus used was so flexible and sensitive that any changes in the rate of respiration of the culture could be detected immediately and measured accurately. The speed with which measurable differences in heat production could be detected when moderate changes in the oxygen tension were made is clearly evident from the figure. Within 5 minutes after the con-

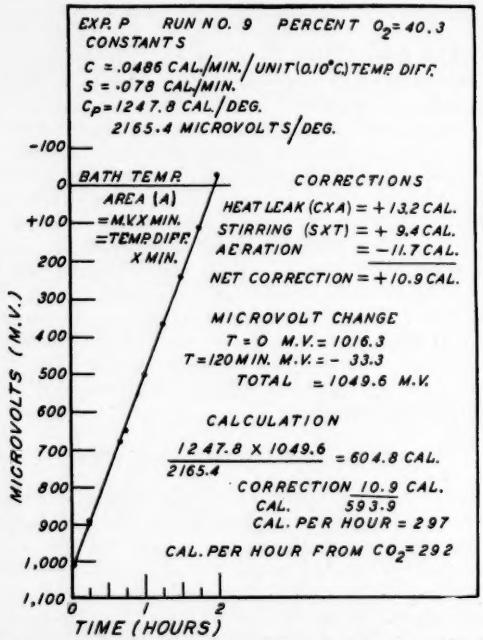


FIGURE 5.—Measurements made and procedure followed in calculating the rate of heat production.

centration of oxygen was changed the temperature response was apparent from the microvolt readings.

In every case where the change made in the partial pressure of oxygen was not more than 100 percent, less than 15 minutes was required for the establishment of a steady state at the new oxygen pressure. This is shown by the fact that the culture would make its total change in the rate of respiration in this short period and then proceed at a constant rate. It is evident from the straight lines obtained over such long periods that conditions were carefully controlled.

Where the partial pressure of oxygen is suddenly reduced to a small fraction of the original pressure, considerable time is required for the culture to reach a steady state at the lower value. In certain experiments the partial pressure of oxygen was suddenly decreased to one-twentieth and in certain experiments to as low as one two-hundredths

of the initial value. It is obvious that considerable time would be necessary even with vigorous aeration for the culture to reach a steady state of respiration. The author determined the minimum time required to reduce the concentration of oxygen from 18 to 0.1

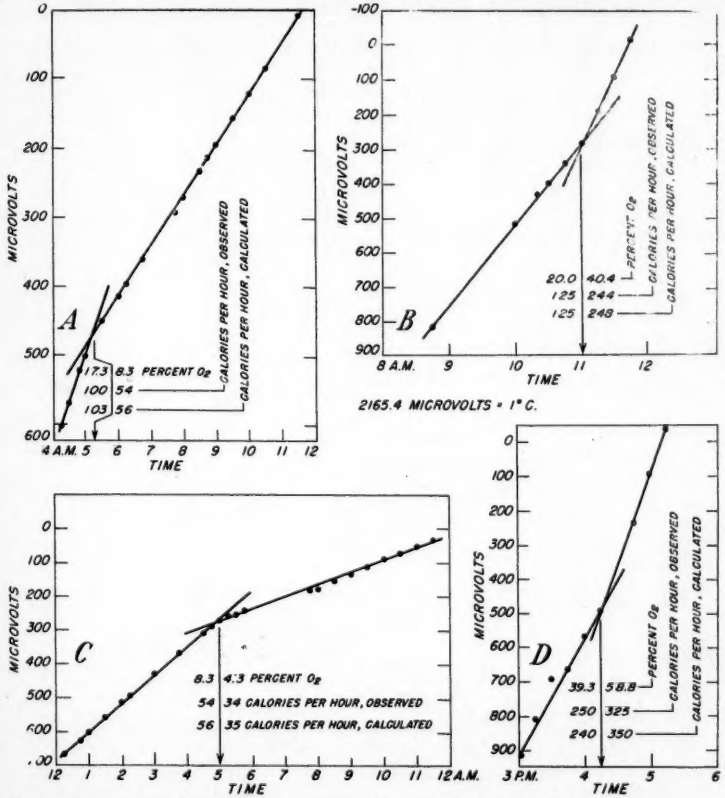
MICROVOLTS
TIME
A
17.3 8.3 PERCENT O2
100 54 CALORIES PER HOUR, OBSERVED
103 56 CALORIES PER HOUR, CALCULATED
4 A.M. 5 6 7 8 9 10 11 12
B
20.0 40.4 PERCENT O2
125 244 CALORIES PER HOUR, OBSERVED
125 248 CALORIES PER HOUR, CALCULATED
8 A.M. 9 10 11 12
2165.4 MICROVOLTS = 1° C.
C
8.3 4.3 PERCENT O2
54 34 CALORIES PER HOUR, OBSERVED
56 35 CALORIES PER HOUR, CALCULATED
12 1 2 3 4 5 6 7 8 9 10 11 12 A.M.
D
39.3 58.8 PERCENT O2
250 325 CALORIES PER HOUR, OBSERVED
240 350 CALORIES PER HOUR, CALCULATED
3 P.M. 4 5 6

FIGURE 6.—Graphs showing the speed with which changes in the rate of respiration of the culture (caused by moderate changes in the partial pressure of oxygen) could be detected with the calorimeter used. The arrow indicates the time the new gas mixture reached the culture. The figures separated by the arrow show the percent of oxygen used and the rate of respiration both calculated and observed. *A*, Experiment N, runs 4 and 5; *B*, experiment R, runs 7 and 8; *C*, experiment N, runs 5 and 6; *D*, experiment O, runs 14 and 15.

percent in the culture by aerating it with a gas mixture containing 0.1 percent oxygen until the rate of respiration became constant.

In one experiment a culture that was free from combined nitrogen was inoculated and aerated for 48 hours with air. The culture was then transferred to the sterile calorimeter, as described, and aerated with a gas mixture containing 18 percent oxygen until the rate of respiration, as measured by both the heat and $CO_2$ evolved, became constant at 105 calories per hour. Nitrogen containing 0.1 percent oxygen was then bubbled through the well-stirred medium at the rate of 14 liters per hour. Heat and $CO_2$ measurements were made to

determine the time required for the culture to reach a constant rate of respiration with the new gas mixture. The data are shown in figure 7*A*.

The heat measurements show that 15 minutes after aeration was begun with nitrogen containing 0.1 percent oxygen the rate of respira-

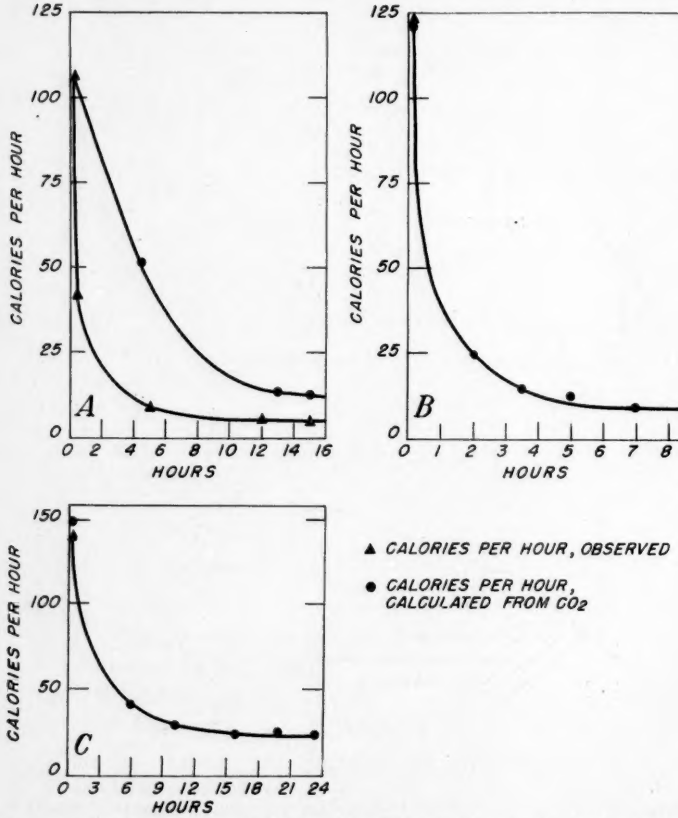


FIGURE 7.—The time required for cultures to reach a steady state with respect to the rate of respiration at the new oxygen pressure when the partial pressure of oxygen is suddenly decreased to a small fraction of the initial pressure: *A*, $N_2$ fixation; *B*, $NH_4$ assimilation; *C*, $NO_3$ assimilation.

tion dropped to only 42 calories per hour, which represents a 60 percent decrease in the first 15 minutes. After 5 hours, the rate of respiration was 4 percent greater than at 15 hours. The rate of respiration did not reach a constant value until at least 10 hours after the 0.1 percent oxygen-gas mixture reached the culture. The final $CO_2$ measurements at 13 and 15 hours confirm the heat measurements. The

obviously high $CO_2$ point at 5 hours was caused by a slight mixing of the last portion of $CO_2$ produced at 18 percent oxygen and that produced at 0.1 percent. The mixing took place in a 20-liter air cushion through which the gas passed after leaving the culture on its way to the $CO_2$ absorbing towers. This air cushion was removed after this test.

In another experiment, a culture assimilating the ammonium ion was allowed to reach a constant rate of respiration (125 calories per hour) at 20 percent oxygen. The culture was then suddenly subjected to nitrogen gas containing 0.1 percent oxygen and the rate of respiration was determined continuously until it reached a constant value. The data obtained are shown in figure 7, *B*. Here, again, the rate of respiration dropped very rapidly during the first 2 hours. It required 7 hours, however, for the culture to reach a constant rate of respiration.

A similar experiment was carried out in which a culture was assimi-

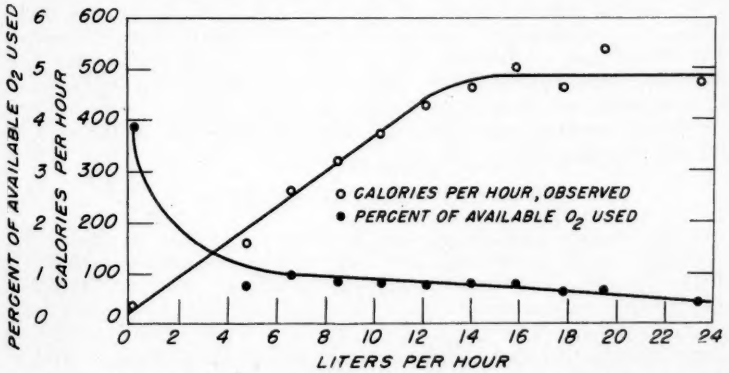


FIGURE 8.—The rate of respiration of *Azotobacter* (in a nitrogen-free medium) with 90 percent oxygen as influenced by the rate of aeration.

lating the nitrate ion. The culture, respiring at a constant rate of 150 calories per hour in 20.4 percent oxygen, was suddenly subjected to nitrogen containing 1 percent oxygen and the rate of respiration was followed continuously until it reached a constant value. The data are shown in figure 7, *C*. Fifteen hours were required for the culture to reach a steady state after the partial pressure of oxygen was reduced to one-twentieth of the initial value.

It is evident that when the partial pressure of oxygen in a culture is suddenly decreased as much as one two-hundredth of the initial pressure considerable time is required for the culture to reach a steady state at the lower oxygen tension even when it is aerated vigorously and stirred mechanically.

## RATE OF AERATION

To demonstrate that the gas-liquid interface supplied adequate oxygen at all times and effectively removed the $CO_2$, the following experiment was carried out: A culture of *Azotobacter*, fixing nitrogen was aerated at different rates varying from 0 to 23.5 liters per hour

with 90 percent oxygen. All the gas passed through the medium which was also mechanically stirred at the normal rate. The rate of heat production was measured for each aeration rate and the oxygen consumed was calculated from the heat produced. The percentage of available oxygen utilized was calculated and is shown by the lower curve of figure 8. The rate of respiration at the different rates of aeration is shown by the upper curve.

The data show clearly that an adequate gas-liquid interface is absolutely necessary if respiration is to proceed at a maximum rate. It is seen that a gas-liquid interface supplied by an aeration rate of 14 liters per hour was required to remove the $CO_2$ and supply the necessary oxygen for a maximum rate of respiration. From the lower curve it is evident that the culture did not suffer from lack of oxygen when the aeration rate was 6 liters per hour, yet the increased gas-liquid interface resulting from the increased rate of aeration caused an increase in the rate of respiration. However, a 67-percent increase in the rate of aeration above 14 liters per hour with a corresponding increase in the gas-liquid interface did not increase the rate of respiration. This shows definitely that at 14 liters per hour the culture had reached a steady state with respect to the rate of respiration. In other experiments with oxygen concentrations below that of the air, the rate of aeration was in many cases doubled with no increase in the rate of respiration.

## RESULTS WITH CALORIMETER

If *Azotobacter* oxidizes glucose to $CO_2$ and water, without the accumulation of intermediate or partly oxidized compounds, the $CO_2$ evolved should give a direct measure of the amount of glucose oxidized and the oxygen consumed. One can then calculate how much heat should be liberated by the amount of $CO_2$ evolved. This amount of energy should be equal to that actually measured in the calorimeter. The amount of energy used for the nitrogen-fixation process is probably insignificant as compared to that liberated as heat.

In order to study the rate of respiration of a culture of *Azotobacter* from the time of inoculation, the calorimeter was assembled and carefully sterilized with a heavy suspension of calcium hypochlorite as described. The sterile medium was inoculated and immediately 1,200 gm. was transferred aseptically to the sterile vacuum flask of unit 2 of the calorimeter. Aeration with 19 percent oxygen was begun at once and heat and $CO_2$ measurements were made continuously for 96 hours. The results of this experiment are shown in figure 9. The measured heat in calories produced per hour and the amount of heat that should have been produced during the same period as calculated from the $CO_2$ evolved are plotted against time.

The rate of air flow through the culture in this experiment was 14 liters per hour, which removed the $CO_2$ as fast as formed. Under these conditions only 0.48 percent of the available oxygen was utilized. As the source of air supply was from compressed air tanks, the partial pressure of oxygen in the system was constant at 0.19 atmosphere throughout the experiment.

The close agreement that exists between the measured and the calculated heat evolved during the entire experiment shows that the glucose was primarily oxidized to carbon dioxide and water (and of

course to the products of cell growth and the other normal products of the reactions of the cells) without a measurable accumulation of intermediate products. It is also evident that under these optimum aerobic conditions, and at this concentration of oxygen, no anaerobic respiration took place, with the result of a respiration quotient of unity.

For the first 8 hours, little or no growth took place. From the eighth to the thirty-sixth hour, the number of living organisms increased rapidly, thus accelerating the rate of heat and $CO_2$ production. After the thirty-sixth hour, the rate of respiration became constant and remained so to the end of the experiment. Here the number of living organisms was constant, as indicated by the constant rate of heat and $CO_2$ production. The curve, moreover, shows clearly the growth phases discussed by Buchanan (*3*).

The results reported in figure 9 serve admirably to show what changes may occur in the efficiency with which the cells utilize oxygen,

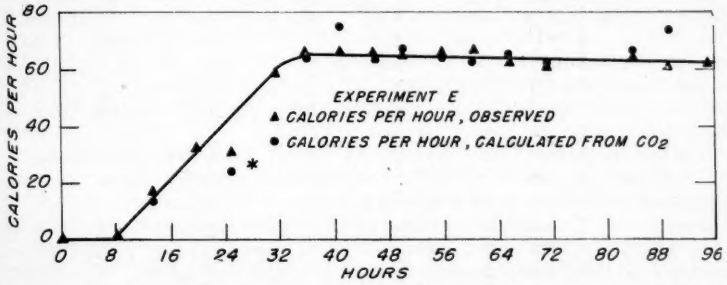


FIGURE 9.—The rate of respiration of *Azotobacter* (while fixing nitrogen) from the time of inoculation, under a partial pressure of 0.19 atmosphere of oxygen. The two points designated by the asterisk were obtained while the mechanical stirrer was being repaired. This decrease in the rate of respiration indicates the absolute necessity of adequate stirring as well as vigorous aeration.

and how the efficiency varies as the age of the culture increases. From inoculation to the thirty-sixth hour, the number of living cells rapidly increased, as shown on the graph by the increasing rate of heat and $CO_2$ production. During this period, the utilization of oxygen per cell was very high, though not necessarily higher than when the number of living cells became constant. After the thirty-sixth hour, the number of living cells apparently reached a maximum and remained constant throughout the experiment, as determined by $CO_2$ and heat measurements.

Although the number of living cells remained constant, new cells were rapidly being formed. (Direct evidence supporting this statement is presented in detail in a later paper (*8*).) This increase in the new cells was offset, however, by the old cells dying at the same rate. During this period (thirty-sixth to ninety-sixth hour) when both the number of living cells and the rate of oxygen consumption (measured by both $CO_2$ and heat production) remained constant, it appears that the efficiency of oxygen consumption per cell is constant and does not decrease with age.

Evidence that only the living cells were responsible for the oxygen consumed and the $CO_2$ evolved was obtained by measurements before and after the culture was killed with mercuric chloride. In one experiment, the observed heat and the heat calculated from the $CO_2$ evolved were 47 and 44 calories per hour, respectively. Upon the addition of mercuric chloride to the culture, the observed heat production fell immediately to zero while the rate of $CO_2$ production was equivalent to only 7 calories per hour. This apparent slow rate of $CO_2$ production after the addition of mercuric chloride was easily accounted for by the fact that considerable time was required to remove completely the last traces of $CO_2$ even with vigorous aeration and stirring. At the end of 3 hours of aeration no more carbon dioxide was evolved.

## DISCUSSION

For a culture of *Azotobacter* to carry on its normal metabolic processes, it must have at all times an adequate amount of oxygen and at the same time the end products must be removed effectively as fast as formed. In certain experiments reported in the literature where few or no precautions were taken to remove the $CO_2$ or to supply an adequate amount of free oxygen in the proper manner, it is to be expected that the behavior of *Azotobacter* would differ from that in the present experiments.

In view of the fact that a respiratory quotient [8] of unity was obtained in these studies under controlled conditions, a review of certain of Bonazzi's experiments is desirable.

Bonazzi (*2*), in apparent agreement with other workers, reports $CO_2 : O_2$ ratios from three experiments of 1.08, 1.07, and 1.09, respectively. Bonazzi's experiment No. 44 (*2, p. 338*) reports that the air above the medium contained 309.4 cc. of $CO_2$ and that 286.2 cc. of oxygen were consumed. The apparent ratio $CO_2 : O_2$ is 1.08 as calculated by Bonazzi. However, all the $CO_2$ produced by the culture was not considered in the calculation. If the partial pressure of $CO_2$ above the medium had been kept near that in air, practically all the $CO_2$ dissolved in the medium would have been found in the gas phase. Titration revealed 50.70 mg. or 25.8 cc. of $CO_2$ in the medium. This makes a total of 335.2 cc. of $CO_2$ produced by the organisms while only 286.2 cc. of oxygen was consumed. The true ratio $CO_2 : O_2$ should be 1.17 and very probably would have been greater if the incubation period had been longer or the medium more heavily buffered. When the total $CO_2$ produced in these experiments is considered, it is clearly evident that intramolecular respiration proceeded under such unfavorable conditions.

Meyerhof and Burk (*13*) present data on the efficiency of oxygen utilization as determined by total respiration and introduce the term "$QO_2$" to represent the total respiration per milligram of dried cells per hour. It was pointed out that the efficiency with which *Azotobacter* cells can utilize oxygen is very high in young cultures, and decreases very rapidly with increasing age of the culture. In these measurements, the dry weight of the total number of cells without

[8] The oxygen consumed was not measured directly but was calculated from the calories of heat evolved according to the reaction mentioned earlier.

distinction between the living and the dead was used in calculating $QO_2$.

In measuring the efficiency of oxygen utilization, care must be taken to credit the oxygen consumption only to the living cells and not to the total number of cells in the culture. In an old culture the total number of cells present is by no means an indication of the number of living cells.

From these considerations, it is obvious that measurements of the rate of oxygen consumption per living cell over the entire life of the culture mean nothing if the total number of cells is determined, because only the living cells are utilizing the oxygen. From this, the results of Meyerhof and Burk are what one would expect and, in fact, what one might easily predict. It is also obvious that the amount of oxygen consumed per unit of dry weight of cells per hour must decrease since dead cells increase in number almost from the beginning and continue to do so throughout the life of the culture. On the other hand, the number of living cells, which alone consume the oxygen, very soon reaches a maximum and then, after a period, begins to decline.

## SUMMARY

A differential calorimeter was rebuilt so that the heat and carbon dioxide liberated by a culture of bacteria could be measured simultaneously over the entire range of oxygen partial pressures from 0.001 to 1.0 atmosphere.

The apparatus was so designed that the culture could be vigorously aerated with any gas mixture. The vigorous aeration, assisted by the rapid mechanical stirring, presented such an enormous gas-liquid interface to the culture that adequate oxygen at the partial pressure in question was insured and at the same time the carbon dioxide was effectively removed as fast as formed.

The heat and carbon dioxide produced could be measured accurately over long periods of time.

The calorimeter was so sensitive that sudden changes in the rate of respiration caused by changes in the partial pressure of oxygen were quickly revealed by the heat measurements.

The experiments presented demonstrate the need of vigorous aeration and stirring to obtain a maximum rate of respiration.

With a constant partial pressure of oxygen approaching that in the air, the growth phases of a culture of *Azotobacter* were accurately followed.

From the carbon dioxide produced and the oxygen consumed (as calculated from the calories of heat evolved) the respiratory quotient of *Azotobacter* was determined when the partial pressure of oxygen was near that in air.

*Azotobacter* produced the maximum amount of heat possible from the oxygen utilized. This was found to be true even in old cultures, which indicates that the efficiency of oxygen utilization per living cell remains constant with increasing age of the culture.

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# RELATIONSHIP OF CERTAIN CHARACTERISTICS OF SEED COTTONS TO GINNING[1]

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## INTRODUCTION

The removal of the lint fibers from cotton seeds by means of gin saws is not purely mechanical in the sense that all variables which may affect the processes are equal for all lots of seed cotton. Ginning performance and efficiency vary not only with the ginning organization and mechanical perfection of the machinery but also with the seed cotton being processed.[2]

The possibility that varietal characteristics may influence the time and energy required to gin unit weights of seed cotton is one of the many problems arising out of the general investigations being conducted by the United States Department of Agriculture to enhance cotton lint quality through selection and breeding, and through improvement of ginning operations.

Differences in ginning performance of several seed cottons were described as early as 1879 by Watson, who was one of the pioneers in cotton ginning research.[3] Watson measured the time and energy consumed in ginning each of a number of widely different cottons. From these studies he found that Indian cotton, Egyptian cotton, and Indian-grown American cotton differed noticeably in their ginning requirements. He states (Vol. 1, p. 61):

> The difference thus shown to exist between the several varieties of cotton in respect of the power consumed in their cleaning can be accounted for in two ways: differences in the strength of the attachment of the fibre to the seed and differences in the roughness of the seed.[4]

Roughness applies to the degree of fuzziness. Watson's statements imply that large, fuzzy seeds remain in the seed roll longer and retard the action of the gin saws.

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[1] Received for publication June 25, 1942. The cotton varieties studied were grown at Stoneville, Miss., through cooperation with the Delta Branch Experiment Station, and the ginning tests were performed at the U. S. Cotton Ginning Laboratory at Stoneville in cooperation with the Bureau of Agricultural Chemistry and Engineering.

[2] BENNETT, C. A., and GERDES, F. L. EFFECTS OF GIN-SAW SPEED AND SEED-ROLL DENSITY ON QUALITY OF COTTON LINT AND OPERATION OF GIN STANDS. U. S. Dept. Agr. Tech. Bul. 503, 40 pp., illus. 1936.

GERDES, F. L., and BENNETT, C. A. EFFECT OF ARTIFICIALLY DRYING SEED COTTON BEFORE GINNING ON CERTAIN QUALITY ELEMENTS OF THE LINT AND SEED AND ON THE OPERATION ON THE GIN STAND. U. S. Dept. Agr. Tech. Bul. 508, 62 pp., illus. 1936.

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[3] WATSON, [J.] F. REPORT ON COTTON GINS, AND ON THE CLEANING AND QUALITY OF INDIAN COTTON 2 v. London. 1879.

[4] Watson uses the word "cleaning" in the same sense that the word "ginning" is now used, to describe the process of removing the fibers from the seed.

Federow, [5] a more recent investigator, makes a theoretical analysis of cotton ginning. He considers strength of fiber attachment to the seed coat as one of the principal factors affecting the difference in power required to gin seed cottons. He estimates the strength of fiber attachment to the seed coat to vary from about 2 to 2.7 gm. per single fiber. Federow states (*p. 4*):

> Now, in the last analysis, the power required for ginning depends upon the amount of work each tooth of the gin saw does in pulling the fibers off the seed coat. Each time the tooth enters the roll it will tear, on the average, 100 fibers from the seed. To do this work, 200 to 270 grammes (.444 to .6 lb.) pulling strength must be exerted.

His analysis also contains statements which suggest that the number of fibers per pound of lint, the percentage of lint, and other factors that determine the number of fibers removed from the seed by each saw tooth passing through the roll may affect the time and the energy consumed in ginning.

Although it is a common opinion among cotton ginners that certain lots of cotton may gin more rapidly than others, no definite information is available to show whether there are marked differences in ginning efficiency among American upland cottons. During the years 1936 and 1937, ginning tests were made at the United States Cotton Ginning Laboratory at Stoneville, Miss., to gain information on this point and to ascertain whether certain seed-cotton properties were responsible for such differences as might be found to exist.

## MATERIALS AND METHODS

Seed cottons for the ginning tests were obtained from special plantings at the Delta Branch Experiment Station, Stoneville, Miss., in 1936 and 1937. The same 16 varieties were planted both years, but in 1937 another variety, Slick-seeded Acala, [6] was added. In 1936, approximately one-eighth acre of each variety was planted; in 1937 the variety plots were increased to approximately one-fourth acre each. In 1936, the varieties were harvested in 3 pickings each, but the entire yield of each variety was thoroughly mixed before it was divided into triplicate ginning lots. In 1937 each variety was harvested in 2 pickings. The yields from each variety were sufficient for 4 ginning tests for each picking or a total of 8 ginning tests for each variety. In all, 184 lots of seed cotton were tested, 48 ($16\times3$) in 1936 and 136 ($17\times8$) in 1937.

A ginning-test lot consisted of 30 pounds of seed cotton weighed after a period of storage and after all varieties were practically uniform with respect to moisture content. The tests were made on a 10-inch diameter, 20-saw gin with a plain front, operated at a saw speed of 570 r. p. m. A separate electric motor was used to drive the saw mandrel, thus making it possible to measure the number of watt-hours of electrical energy required to drive the saws only during each test. The electrical energy consumed was measured by means of a kilowatt-hour meter of standard type but designed for small readings. It was found that the most satisfactory method of reading the meter was to count the number of revolutions of the aluminum disk mounted on the main shaft of the meter. The disk was marked off into eight

[5] FEDEROW, W. S. COTTON GINNING RESEARCH. Cotton and Cotton Oil News 34 (20): 3-4, 1933.
[6] This cotton is not a commercial variety.

equal divisions and the readings were made to one-eighth of a revolution of the disk. One revolution of the disk was equivalent to 4 watt-hours.

In performing the tests, an effort was made to eliminate or control all variables that might affect the ginning time or the energy consumed except the inherent properties of the seed cottons representing the several varieties. Friction variation was eliminated to some extent by allowing the machinery to run idle each morning before starting the tests until two successive readings of the watt-hour meter indicated that the energy required to overcome the friction of the machinery had reached a practically constant figure. The order of ginning the different varieties in each series was determined by lot.

Before a test was started, 10 pounds of seeds from previously ginned cotton of the variety to be tested were placed in the roll box. A handful from the 30-pound sample of seed cotton was mixed with this seed roll to insure good operation. The breast was then lifted from the saws and the motor was started. When the saws had accelerated to the required speed, and at a signal from the timekeeper, the breast was lowered and ginning commenced. With a stop watch, the timekeeper measured the ginning time which began when the gin breast was lowered to the revolving saws and ended when the seed roll ceased rotating at the end of the test. The timekeeper also counted the number of revolutions of the disk of the watt-hour meter during the same period of time.

The 30-pound seed-cotton sample was fed by hand and at such a rate that the seed roll remained very soft and at approximately a constant density throughout the test. Securing and maintaining a uniform seed roll density for every test was especially important. The personal element entered into this, however, because the density was determined by the gin operator on the basis of observation and "feel." Ginning was continued on each lot of cotton until the seed roll became too soft to rotate. This gave a uniform cleaning of the seeds for different varieties and a definite stopping point for the measurement of the ginning time.

The total watt-hours consumed during each test includes the amount of electrical energy needed to run the machinery alone as well as the amount required to gin the cotton. The net energy that would have been required to turn the unloaded machinery was computed by counting the revolutions of the meter disk while running the machinery idle for 1 minute immediately preceding and for 1 minute immediately following each test, by averaging the two readings, and by multiplying the average by the number of minutes the test consumed. The net energy required to run the machinery subtracted from the total energy gives the net energy consumed in ginning the cotton.

Data obtained from each test were: (1) Time required to gin 30 pounds of seed cotton; (2) watt-hours of electricity consumed in driving the saws during the test, (3) watt-hours of electricity consumed in driving the saws when not ginning for periods of 1 minute before and after each test; and (4) weights of lint, seeds, and mote waste. From these data were obtained: (1) Time required to gin enough seed cotton to produce 10 pounds of lint; (2) net energy in watt-hours consumed in ginning 30 pounds of seed cotton and 10 pound of lint; and (3) lint percentage.

Samples of seed cotton, lint, and seeds were taken for moisture determinations and for laboratory measurements of their respective properties. The lint index, seed index (weight of 100 seeds), and the percentage of fuzz fiber by weight on the seeds were obtained each year for each variety. Measurements of strength of fiber attachment to the seeds were made for each variety of the 1936 crop.

## RESULTS AND CONCLUSIONS

The data concerning the time and energy consumed in ginning unit weights of seed cotton were, on the whole, consistent for the 2 crop years. Because of the larger number of replications for the 1937 crop, however, more reliance can be placed upon the data for that year than upon the data for the 1936 crop.

Each year appreciable differences were found between varieties in the average time required to gin them (tables 1, 2, and 3). The varieties tended to behave consistently throughout the series of tests for each year and those varieties that ginned most rapidly in 1936 tended to do likewise in 1937. The average time for 30 pounds of seed cotton from the 16 different varieties ranged from 9.7 to 13.0 minutes in 1936 and from 10.3 to 16.8 minutes in 1937. Slick-seeded Acala, a variety tested only in 1937, required the least time of all, 7.9 minutes.

TABLE 1.—*Mean time required and mean energy consumed in ginning seed cotton and lint from each of 16 varieties grown at Stoneville, Miss., 1936*[1]

| Variety | Ginning time required for 30 pounds of seed cotton | Ginning time required for enough cotton to yield 10 pounds of lint | Total energy consumed in ginning 30 pounds of seed cotton | Net energy consumed in ginning 30 pounds of seed cotton | Net energy consumed in ginning enough cotton to yield 10 pounds of lint |
|---|---|---|---|---|---|
| | *Minutes* | *Minutes* | *Watt-hours* | *Watt-hours* | *Watt-hours* |
| Acala (Rogers) | 9.7 | 9.2 | 199.6 | 90.0 | 84.4 |
| Arkansas No. 17 | 9.8 | 10.7 | 206.0 | 95.2 | 103.6 |
| Cleveland (Wannamaker) | 11.2 | 11.0 | 225.2 | 95.6 | 93.6 |
| Cook No. 912 | 10.6 | 11.0 | 211.2 | 90.4 | 94.0 |
| Delfos No. 4 | 12.5 | 12.9 | 235.2 | 95.6 | 99.6 |
| Deltapine | 10.8 | 9.7 | 218.0 | 94.8 | 85.6 |
| Dixie Triumph No. 759 | 9.7 | 10.5 | 196.0 | 87.2 | 94.4 |
| Farm Relief No. 2 | 13.0 | 12.7 | 257.2 | 110.8 | 107.6 |
| Half and Half | 12.4 | 10.0 | 230.8 | 90.8 | 114.8 |
| Mexican Big Boll | 12.4 | 12.3 | 242.8 | 104.0 | 103.6 |
| Qualla | 11.7 | 10.0 | 225.6 | 100.0 | 81.2 |
| Rowden No. 2088 | 12.3 | 12.5 | 241.2 | 109.2 | 110.8 |
| Startex No. 619 | 10.7 | 10.4 | 211.6 | 92.4 | 88.0 |
| Stoneville No. 5 | 10.9 | 11.0 | 218.8 | 95.6 | 97.6 |
| Triumph No. 44 | 10.1 | 10.8 | 200.0 | 89.2 | 95.6 |
| Wilds No. 5 | 10.9 | 12.5 | 228.8 | 105.6 | 120.4 |
| Standard error of difference between means of varieties | .57 | .54 | 8.96 | 4.67 | 4.32 |
| Least difference between means of varieties significant at odds of 99 to 1 | 1.57 | 1.48 | 24.64 | 12.84 | 11.88 |

[1] Mean of 3 tests.

TABLE 2.—*Mean time required and mean energy consumed in ginning seed cotton and lint from each of 17 varieties grown at Stoneville, Miss., 1937* [1]

| Variety | Ginning time required for 30 pounds of seed cotton | Ginning time required for enough cotton to yield 10 pounds of lint | Total energy consumed in ginning 30 pounds of seed cotton | Net energy consumed in ginning 30 pounds of seed cotton | Net energy consumed in ginning enough cotton to yield 10 pounds of lint |
|---|---|---|---|---|---|
| | *Minutes* | *Minutes* | *Watt-hours* | *Watt-hours* | *Watt-hours* |
| Acala (Rogers) | 11.1 | 10.2 | 198.0 | 64.4 | 59.6 |
| Acala (Slick-seeded) | 7.9 | 7.6 | 152.4 | 56.0 | 53.6 |
| Arkansas No. 17 | 12.4 | 12.4 | 216.8 | 69.6 | 71.2 |
| Cleveland (Wannamaker) | 13.6 | 12.9 | 233.2 | 67.6 | 64.0 |
| Cook No. 912 | 12.5 | 12.3 | 218.0 | 67.2 | 67.6 |
| Delfos No. 4 | 14.0 | 13.5 | 242.8 | 74.0 | 71.6 |
| Deltapine | 13.3 | 11.3 | 237.6 | 72.8 | 61.6 |
| Dixie Triumph No. 759 | 11.7 | 11.5 | 211.6 | 67.6 | 66.4 |
| Farm Relief No. 2 | 16.8 | 15.4 | 297.2 | 93.6 | 86.0 |
| Half and Half | 13.0 | 10.3 | 228.4 | 72.0 | 57.2 |
| Mexican Big Boll | 13.8 | 13.1 | 244.0 | 76.0 | 72.4 |
| Qualla | 12.9 | 11.2 | 224.4 | 68.8 | 59.6 |
| Rowden No. 2088 | 15.7 | 15.2 | 272.8 | 80.8 | 78.4 |
| Startex No. 619 | 10.3 | 9.9 | 186.0 | 59.2 | 56.4 |
| Stoneville No. 5 | 11.6 | 10.8 | 209.2 | 67.2 | 62.4 |
| Triumph No. 44 | 12.3 | 12.0 | 220.0 | 68.8 | 67.6 |
| Wilds No. 5 | 13.5 | 13.9 | 243.6 | 76.8 | 79.2 |
| Standard error of difference between means of varieties | .26 | .24 | 4.53 | 1.71 | 1.57 |
| Least difference between means of varieties significant at odds of 99 to 1 | .68 | .63 | 11.90 | 4.49 | 4.12 |

[1] Mean of 8 tests.

TABLE 3.—*Results of variance analyses of data concerning the time (minutes) and—energy (watt-hours) required to gin 30 pounds of seed cotton and 10 pounds of lint for 16 varieties grown in 1936, and 17 varieties grown in 1937 at Stoneville, Miss.*

1936

| Source of variance | Degrees of freedom | Mean square | | | | |
|---|---|---|---|---|---|---|
| | | Time required to gin 30 pounds of seed cotton | Time required to gin enough cotton to yield 10 pounds of lint | Total energy consumed in ginning 30 pounds of seed cotton | Net energy consumed in ginning 30 pounds of seed cotton | Net energy consumed in ginning enough cotton to yield 10 pounds of lint |
| Varieties | 15 | 3.49** | 4.05** | 912.44** | 156.72** | 406.96** |
| Series | 2 | 1.18 | .96 | 547.84* | 534.56** | 342.96** |
| Error | 30 | .49 | .44 | 120.32 | 32.72 | 27.92 |
| Total | 47 | 1.48 | 1.61 | 391.31 | 93.65 | 162.30 |

1937

| | | | | | | |
|---|---|---|---|---|---|---|
| Varieties | 16 | 31.86** | 31.68** | 8,422.68** | 579.00** | 634.40** |
| Harvestings | 1 | 7.06** | 43.82** | 1,574.88** | 64.00* | 908.96** |
| Variety×harvestings | 16 | .52* | .44* | 146.48* | 24.00* | 21.92** |
| Series within harvestings | 6 | 1.29** | 1.22** | 396.04** | 101.32** | 97.64** |
| Error | 96 | .28 | .24 | 82.00 | 11.68 | 9.88 |
| Total | 135 | 4.15 | 4.36 | 1,103.17 | 84.74 | 95.89 |

*Significant at the 5-percent level; **significant at the 1-percent level.

The ginning time on the basis of 10 pounds of lint gave values practically as widely dispersed and as consistent as the values for 30 pounds of seed cotton (tables 1 and 2). The respective order of varieties in regard to rapidity of ginning, however, was changed considerably because of differences in lint percentages (tables 4 and 5).

Variance analyses of the data show that for each year there are significant differences among the varieties in the time required to gin either 30 pounds of seed cotton or enough seed cotton to produce 10 pounds of lint (tables 1, 2, and 3). In general, a difference of 1.57 minutes in the ginning time for 30 pounds of seed cotton, and 1.48 minutes in the ginning time for 10 pounds of lint, between any two variety means of the 1936 crop, can be considered highly significant (odds 99:1). Similarly, a difference of 0.68 minute in the ginning time for 30 pounds of seed cotton, and 0.63 minute in the ginning time for 10 pounds of lint, between any two variety means of the 1937 crop can be considered highly significant.

TABLE 4.—*Seed-cotton properties of the varieties grown at Stoneville, Miss., in 1936*

| Variety | Seed index [1] | Fuzz fibers by weight [2] | Lint [3] | Number of fibers in 30 pounds of seed cotton | Mean strength of fiber attachment to seed [4] | Mean strength of fiber attachment × number of fibers in 30 pounds seed cotton |
|---|---|---|---|---|---|---|
| | *Grams* | *Percent* | *Percent* | *Hundred thousands* | *Grams* | *Hundred kilograms* |
| Acala (Rogers) | 11.1 | 9.2 | 35.3 | 13,367 | 1.278 | 17,083 |
| Arkansas No. 17 | 10.7 | 10.1 | 30.7 | 9,723 | 1.397 | 13,583 |
| Cleveland (Wannamaker) | 10.5 | 14.0 | 34.3 | 11,680 | 1.125 | 13,140 |
| Cook No. 912 | 8.8 | 11.0 | 32.0 | 12,715 | 1.202 | 15,283 |
| Delfos No. 4 | 10.2 | 11.5 | 32.3 | 10,780 | 1.169 | 12,602 |
| Deltapine | 9.4 | 11.9 | 37.0 | 13,040 | 1.107 | 14,435 |
| Dixie Triumph No. 759 | 9.9 | 10.3 | 30.7 | 10,266 | 1.382 | 14,188 |
| Farm Relief No. 2 | 11.8 | 15.6 | 34.3 | 11,260 | 1.340 | 15,088 |
| Half and Half | 9.1 | 9.6 | 41.0 | 14,785 | 1.229 | 18,171 |
| Mexican Big Boll | 12.2 | 12.9 | 33.7 | 11,499 | 1.254 | 14,420 |
| Qualla | 11.3 | 11.3 | 39.0 | 12,790 | 1.153 | 14,747 |
| Rowden No. 2088 | 11.9 | 13.8 | 32.7 | 9,424 | 1.438 | 13,552 |
| Startex No. 619 | 11.0 | 8.6 | 34.3 | 11,213 | 1.736 | 19,466 |
| Stoneville No. 5 | 9.8 | 11.4 | 33.0 | 12,888 | 1.436 | 18,507 |
| Triumph No. 44 | 10.6 | 11.3 | 31.0 | 10,799 | 1.275 | 13,769 |
| Wilds No. 5 | 12.0 | 10.6 | 29.3 | 11,097 | 1.138 | 12,628 |
| Average | 10.6 | 11.4 | 33.8 | 11,708 | 1.291 | 15,041 |

[1] Average of 10 samples of 100 seeds.
[2] Average of 3 determinations.
[3] Average of 3 ginning tests.
[4] Average of 256 fibers from each of 16 seeds.

Most of the foregoing discussion with regard to time applies equally well to energy. Varieties differed considerably as to the total amount of energy (watt-hours), as well as the net energy required to gin them. The varieties tended to behave consistently throughout the series of tests for each year, and those that required the most energy for ginning either 30 pounds of seed cotton or 10 pounds of lint in 1936 did likewise in 1937 (tables 1 and 2). As in the case of time, the amount of energy required to gin Slick-seeded Acala was least of all.

Variance analyses of the data showed that in both 1936 and 1937 there were statistically significant differences among varieties in both total and net energy consumption (tables 1, 2, and 3). In general, a difference of 12.84 watt-hours in net energy required to gin 30 pounds of seed cotton, or 11.88 watt-hours in net energy required to gin

TABLE 5.—*Seed-cotton properties of the varieties grown at Stoneville, Miss., in 1937*

| Variety | Seed index | | | Fuzz fibers by weight | | | Lint | | |
|---|---|---|---|---|---|---|---|---|---|
| | First picking [1] | Second picking [1] | Average | First picking [2] | Second picking [2] | Average | First picking [3] | Second picking [3] | Average |
| | *Grams* | *Grams* | *Grams* | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* |
| Acala (Rogers) | 13.9 | 14.0 | 14.0 | 8.7 | 8.8 | 8.8 | 37.0 | 35.3 | 36.2 |
| Acala (Slick-seeded) | 14.4 | 14.5 | 14.4 | 5.2 | 5.1 | 5.2 | 36.0 | 34.0 | 35.0 |
| Arkansas No. 17 | 12.5 | 12.6 | 12.6 | 9.8 | 9.5 | 9.6 | 33.3 | 31.7 | 32.5 |
| Cleveland (Wannamaker) | 12.5 | 12.8 | 12.6 | 12.3 | 13.0 | 12.6 | 36.7 | 33.7 | 35.2 |
| Cook No. 912 | 10.9 | 10.7 | 10.8 | 10.0 | 10.3 | 10.2 | 35.0 | 33.0 | 34.0 |
| Delfos No. 4 | 11.8 | 12.2 | 12.0 | 11.6 | 11.4 | 11.5 | 35.3 | 33.7 | 34.5 |
| Deltapine | 10.9 | 11.0 | 11.0 | 10.4 | 11.3 | 10.8 | 40.7 | 38.3 | 39.5 |
| Dixie Triumph No. 759 | 11.4 | 11.3 | 11.4 | 8.5 | 9.2 | 8.8 | 35.0 | 33.0 | 34.0 |
| Farm Relief No. 2 | 14.3 | 14.7 | 14.5 | 14.6 | 13.7 | 14.2 | 37.0 | 35.3 | 36.2 |
| Half and Half | 11.1 | 11.3 | 11.2 | 9.2 | 9.5 | 9.4 | 43.3 | 40.7 | 42.0 |
| Mexican Big Boll | 14.5 | 14.3 | 14.4 | 11.6 | 10.7 | 11.2 | 36.0 | 34.0 | 35.0 |
| Qualla | 14.7 | 14.9 | 14.8 | 11.4 | 10.7 | 11.0 | 40.0 | 37.0 | 38.5 |
| Rowden No. 2088 | 14.2 | 13.9 | 14.0 | 13.1 | 12.9 | 13.0 | 35.3 | 33.7 | 34.5 |
| Startex No. 619 | 13.0 | 12.9 | 13.0 | 8.1 | 7.9 | 8.0 | 36.0 | 34.0 | 35.0 |
| Stoneville No. 5 | 11.0 | 11.3 | 11.2 | 11.2 | 11.2 | 11.2 | 37.3 | 34.3 | 35.8 |
| Triumph No. 44 | 12.3 | 12.1 | 12.2 | 10.6 | 10.2 | 10.4 | 34.7 | 33.0 | 33.8 |
| Wilds No. 5 | 15.1 | 15.0 | 15.0 | 9.6 | 9.0 | 9.3 | 33.7 | 31.0 | 32.4 |
| Average | 12.9 | 12.9 | 12.9 | 10.3 | 10.3 | 10.3 | 36.6 | 34.5 | 35.5 |

[1] Average of 8 samples of 100 seeds each.
[2] Average of 3 determinations.
[3] Calculated from the mean seed index and the mean yield of lint (4 determinations).

10 pounds of lint, between the means of any two varieties of the 1936 crop can be considered highly significant. For similar significance, variety means of the 1937 crop must differ 4.49 watt-hours in the net energy required to gin 30 pounds of seed cotton and 4.12 watt-hours in net energy required to gin 10 pounds of lint.

There was considerable difference between the samples for the 2 years in the time and energy consumed. On the whole, the samples from the 1936 crop were ginned faster and required more net energy than those of 1937. A part of the difference in ginning requirements between the 2 years might be attributed to differences in seed cotton properties, but it is more probable that the greater part is due to variations in gin-stand operations, such as rate of feed and density of the seed roll. These variables were controlled by the gin-stand operator and were subject to error of judgment. The reasonableness of this explanation for differences in time and energy consumption between the 2 years is substantiated by the significant differences in ginning time and energy requirements between series within the same year (table 3). Differences between series are most logically assigned to variations in gin-stand operations. Since gin-stand operations would certainly vary more between years than between series of the same year, it is equally logical to assign a large part of the differences between years in ginning requirements to variations between the 2 years in ginning operation.

After having established that there are significant differences among varieties in the time and energy required to gin them, the next problem was to ascertain whether these differences could be explained by varietal differences in certain seed-cotton properties. Seed size (seed index), lint percentage, and amount of fuzz were the three properties selected for study (tables 4 and 5). The data were analyzed for covariance.

In order to combine the data for the 2 years into one analysis, it was necessary to omit the figures for Slick-seeded Acala since data for only 1 year were obtained for that variety. Too, since the number of tests made upon each variety were not the same for each year and since the seed-cotton properties were not measured for each ginning test separately, but for the variety as a whole, the covariance was determined on the variety means. Thus the test for significance falls upon the variety × year interaction (table 6).

TABLE 6.—*Mean squares for ginning and seed-cotton variables for 16 varieties grown at Stoneville, Miss., in 1936 and 1937*

| Source of variance | Degrees of freedom | Independent variables | | | Dependent variables | | | |
|---|---|---|---|---|---|---|---|---|
| | | Seed index | Lint percentage | Percentage of fuzz | Time—30 pounds of seed cotton | Time—10 pounds of lint | Energy—30 pounds of seed cotton | Energy—10 pounds of lint |
| Total | 31 | 2.79 | 8.63 | 3.21 | 2.75 | 2.36 | 216.47 | 308.91 |
| Years | 1 | 36.98 | 26.46 | 5.36 | 27.75 | 10.81 | 5,000.00 | 6,418.44 |
| Within years | 30 | 1.65 | 8.04 | 3.14 | 1.92 | 2.08 | 57.02 | 105.26 |
| Varieties [1] | 15 | 3.12 | 15.57 | 6.14 | 3.27 | 3.76 | 102.86 | 193.07 |
| Variety×year | 15 | .17 | .50 | .14 | .57 | .40 | 11.18 | 17.02 |

[1] Significant values of $F$ for varieties $= \frac{\text{variety}}{\text{variety} \times \text{year}} = 2.43$ at the 5-percent level; 3.56 at the 1-percent level.

Beta regression, and simple and multiple correlation coefficients, were calculated for the data as a whole and for the different sources of variance (table 7).

For the data as a whole the sizes of the simple correlation coefficients indicate that there is a significant tendency for an increase in the amount of fuzz upon the seed to be accompanied by an increase in the amount of time and energy consumed in ginning. Also, there is a definite tendency for the larger seeded cottons to require more time than the smaller seeded ones. However, the relationship between seed index and the amount of energy required is negative. This negative correlation coefficient is misleading. It results from the fact that the larger seeded crop of 1937 consumed less energy than the smaller seeded crop of 1936. But it has been pointed out previously that the difference between the 2 years in the amount of energy consumed is probably due to uncontrolled variations in gin-stand operations. This difference between the 2 years in energy consumed is so great that it has an enormous influence on the "over-all" correlation, and results in giving a false indication of relationships. If the variance due to years is eliminated from the data, leaving the "within years" variance, the relationship between seed index and energy consumed is positive; that is, within each year an increase in seed size is accompanied by an increase in the amount of energy consumed.

For the data as a whole, lint percentage has a significant influence only upon the amount of energy required to gin 10 pounds of lint. The correlation is negative. Cottons with high lint percentages require less energy to yield 10 pounds of ginned lint than cottons with low lint percentages.

Since there is little correlation among the seed-cotton properties, the beta coefficients for the "total" differ little from the simple correlation coefficients.

TABLE 7.—*Results of covariance analysis with simple and multiple correlation coefficients* [1] *and beta regression coefficients representing the relationship between the time and energy required to gin either 30 pounds of seed cotton or 10 pounds of lint, and seed index, lint percentage, and percentage of fuzz for 16 varieties grown at Stoneville, Miss., in 1936 and 1937*

| Source of variance | Degrees of freedom | Simple correlation coefficients | | | | Simple correlation coefficients among independent variables | | | Beta coefficients of independent variables | | | Multiple correlation coefficients | Standard error of estimate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Dependent variable | Seed index | Lint percentage | Percentage of fuzz | Seed index and lint percentage | Seed index and percentage of fuzz | Lint percentage and percentage of fuzz | Seed index | Lint percentage | Percentage of fuzz | | |
| Total | 31 | Time—30 pounds of seed cotton | 0.524 | 0.339 | 0.448 | 0.062 | 0.049 | −0.068 | 0.542 | 0.336 | 0.444 | 0.793 | 1.06 |
| | | Time—10 pounds of lint | .562 | −.249 | .501 | | | | .555 | −.252 | .456 | .777 | 1.02 |
| | | Energy—30 pounds of seed cotton | −.362 | −.280 | .550 | | | | −.375 | −.218 | .553 | .708 | 10.94 |
| | | Energy—10 pounds of lint | −.333 | −.624 | .466 | | | | −.319 | −.577 | .442 | .819 | 10.61 |
| Years | 1 | | | | | | | | | | | | |
| Within years (varieties+varieties × years) | 30 | Time—30 pounds of seed cotton | .339 | .204 | .726 | −.201 | .273 | .005 | .206 | .242 | .668 | .777 | .92 |
| | | Time—10 pounds of lint | .445 | −.422 | .656 | | | | .203 | −.384 | .603 | .806 | .90 |
| | | Energy—30 pounds of seed cotton | .533 | −.016 | .712 | | | | .378 | .057 | .608 | .797 | 4.81 |
| | | Energy—10 pounds of lint | .484 | −.673 | .494 | | | | .240 | −.627 | .432 | .867 | 5.39 |
| Varieties | 15 | Time—30 pounds of seed cotton | .369 | .194 | .827 | −.185 | .284 | .012 | .191 | .219 | .770 | .866 | .71 |
| | | Time—10 pounds of lint | .461 | −.458 | .721 | | | | .190 | −.432 | .673 | .878 | .73 |
| | | Energy—30 pounds of seed cotton | .601 | −.044 | .764 | | | | .423 | .026 | .643 | .864 | 4.10 |
| | | Energy—10 pounds of lint | .508 | −.689 | .512 | | | | .262 | −.647 | .446 | .899 | 4.82 |
| Varieties × years | 15 | Time—30 pounds of seed cotton | .096 | .411 | −.498 | −.000 | .003 | −.276 | .450 | .587 | −.337 | .673 | .62 |
| | | Time—10 pounds of lint | .260 | .133 | −.455 | | | | .435 | .290 | −.377 | .569 | .58 |
| | | Energy—30 pounds of seed cotton | −.313 | .454 | −.111 | | | | −.062 | .419 | .005 | .459 | 3.52 |
| | | Energy—10 pounds of lint | .152 | −.448 | .211 | | | | −.166 | −.529 | .065 | .525 | 3.92 |

[1] Significant values of correlation coefficients:

| Degrees of freedom: | *At the 5 percent level* | *At the 1 percent level* |
|---|---|---|
| | *r* | |
| 30 | 0.349 | 0.449 |
| 29 | .355 | .436 |
| 14 | .497 | .623 |
| | *R* | |
| 28 | .490 | .573 |
| 27 | .498 | .582 |
| 12 | .683 | .773 |

The correlation coefficients of greatest interest, however, are those that represent the extent to which varietal differences in time and energy consumed are related to varietal differences in the three seed-cotton properties being considered. These coefficients were obtained for varieties taken as a whole and for varieties "within years." This latter represents the variance remaining in the data after that associated with yearly differences has been eliminated. The "within years" analysis is of special interest because of the very great differences between the 2 years in the amount of energy consumed.

The coefficients for varieties taken as a whole or "within years" are very similar and statements regarding one apply equally well to the other. Since there is some degree of correlation among the seed-cotton properties, the relative importance of each independent variable can best be ascertained by comparing the beta coefficients.

The simple correlation coefficients show that there is a significant tendency for varieties with large seeds and a high percentage of fuzz to require more time and energy to gin them than do varieties with smaller or less fuzzy seeds. Apparently large seeds or seeds with a heavy covering of fuzz are not discharged as rapidly from the gin-roll box during ginning as are smaller seeds or seeds with less fuzz, and consequently the larger or more fuzzy seeded varieties require more time and energy in the ginning process. The beta coefficients show that the percentage of fuzz is the more important of these two seed-cotton properties, especially in determining the amount of time consumed—seed size apparently having more influence upon the amount of energy consumed than upon the time required to gin unit weights of seed cotton or to yield unit weights of ginned lint.

According to the simple correlation coefficients, there is little or no relation between lint percentage and the time and energy required to gin 30 pounds of seed cotton, but there is a significant relation between lint percentage and the time and energy required to gin enough seed cotton to produce 10 pounds of lint. This relationship is negative, the varieties with a high lint percentage requiring less time and energy to yield 10 pounds of lint than do those with a low lint percentage. The beta coefficients indicate that lint percentage is more important than seed size but less important than the amount of fuzz in its effect upon the amount of time consumed in ginning either 30 pounds of seed cotton (positive effect) or 10 pounds of lint; that it has practically no influence upon the amount of energy required to gin 30 pounds of seed cotton; but that it is the most important of all three factors in its effect upon the amount of energy required to gin 10 pounds of lint.

Correlation coefficients for the variety × year interactions are on the whole relatively insignificant, indicating that tendencies which certain varieties may have shown to require relatively more energy or time in 1 year than in the other cannot be related to differences in any of the seed-cotton properties. It is true that a few of the correlation coefficients indicate some degree of relationship, but it must be borne in mind that technical variations which were not completely controlled may confuse the true relationships.

The strength of fiber attachment to the seed was determined for the 1936 crop, and significant differences were found between varieties in the force required to detach fibers from the seed. [7] But differences in

[7] SMITH, W. S., and PEARSON, N. L. A METHOD OF MEASURING THE STRENGTH OF ATTACHMENT OF COTTON FIBERS TO THE SEED AND SOME RESULTS OF ITS APPLICATION. United States Agr. Market Serv., and Bur. Plant Indus., 22 pp., illus. 1941. [Processed.]

mean strength of fiber attachment to the seed were found not to be related to corresponding differences in the amount of energy required to gin these particular cottons. However, in considering the question of strength of fiber attachment in relation to ginning, it is necessary to take into account the total number of fibers which must be detached, and the total force which would be required to detach all of them. This total force was calculated for each variety by multiplying the calculated number of fibers in 30 pounds of seed cotton by the mean strength of fiber attachment. The multiple correlation coefficients and the beta coefficients were then calculated to show the combined and individual effects of seed index, percentage of fuzz, and total force required to detach the fibers upon the energy required to gin the cottons (table 8). The $R$ value obtained was significant, but the beta coefficients indicated that the total force required to detach the fibers had, in the case of these particular varieties, no effect upon the energy consumed. For this reason, the strength of fiber attachment was not measured for the 1937 crop.

The first and second pickings of the 1937 crop were ginned separately and showed significant differences in ginning behavior (table 3). The second picking required significantly more time and energy than the first. It contained more trash, which would account, in part at least, for the greater amount of time and energy consumed. It also had a lower lint percentage (table 5), which in addition would bring about an increase in the time and energy required to gin 10 pounds of lint.

TABLE 8.—*Multiple correlation and beta regression coefficients showing relation between the energy required to gin 30 pounds of seed cotton and the seed index, percentage of fuzz, and mean strength of fiber attachment × number of fibers in 30 pounds of seed cotton, for 16 varieties grown in 1936*

| Dependent variable | $R$ [1] | Beta cofficients of independent variables | | |
|---|---|---|---|---|
| | | Seed index | Percentage of fuzz by weight | Mean strength of fiber attachment × number of fibers |
| Energy required to gin 30 pounds of seed cotton, 1936 | 0.903 | +0.572 | 0.531 | 0.071 |

[1] Significant values of $R$: at the 5-percent level=0.683; at the 1-percent level=0.773.

## SUMMARY

Tests were conducted to ascertain whether certain American upland cottons differed in respect to the time and energy required for ginning and, if so, to ascertain whether variations in certain seed-cotton properties were responsible for these differences.

Plantings of 16 varieties in both 1936 and 1937, and a seventeenth in 1937 only, provided seed cotton for 184 test lots of 30 pounds each—3 for each variety in 1936 and 8 for each variety in 1937.

Varieties were found to differ significantly in the time and net energy required to gin either 30 pounds of seed cotton or enough seed cotton to produce 10 pounds of lint.

The larger and more fuzzy-seeded cottons required more time and energy to gin than the smaller and less fuzzy-seeded varieties. Apparently the large and fuzzy seeds are not discharged from the roll box during ginning as rapidly as smaller and less fuzzy seeds.

Varieties with a high lint percentage required less time and energy to gin 10 pounds of lint than varieties with low lint percentage. Lint percentage, however, had little effect upon the energy required to gin 30 pounds of seed cotton, but did affect the amount of time consumed, an increase in lint percentage tending to be accompanied by an increase in the amount of time required.

The order of influence of the three seed-cotton properties follows. Time required to gin 30 pounds of seed cotton or 10 pounds of lint: (1) Percentage of fuzz, (2) lint percentage, and (3) seed size. Net energy required to gin 30 pounds of seed cotton: (1) Amount of fuzz, (2) seed size, and (3) lint percentage. Net energy required to gin 10 pounds of lint: (1) Lint percentage, (2) amount of fuzz, and (3) seed size.

Strength of fiber attachment to the seed had no effect upon the energy required to gin these particular cottons.

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